SUBSTRATE AVAILABILITY AND SELECTIVITY CONTRIBUTE TO MICROHABITAT SPECIALIZATION IN TWO CENTRAL AMERICAN SEMIAQUATIC ANOLES

NICHOLAS C. HERRMANN[1]

ABSTRACT. Both substrate availability and animal selectivity can contribute to patterns of animal substrate use. In the West Indies, where *Anolis* lizard ecomorphs are specialized for particular microhabitats, the relative influences of availability and selectivity on anole substrate use can change depending on the species and its location. Whether substrate availability and selectivity both contribute to microhabitat specialization in anoles outside the West Indies is largely unexplored. Two species of Central American semiaquatic anoles appear adapted for locomotion on different substrates—*Anolis oxylophus* on wood and leaves and *Anolis aquaticus* on rocks. I evaluated the contributions of substrate availability and selectivity to microhabitat specialization in these two species by comparing their substrate use to substrate availabilities in their stream macrohabitats. Both species selectively avoided the ground and perched instead on elevated substrates, yet the available elevated substrates differed between streams. Specifically, wood was most abundant in streams occupied by *A. oxylophus*, and rocks were most abundant in streams occupied by *A. aquaticus*. As is true with several West Indian anole ecomorphs, both substrate availability and selectivity contribute to microhabitat specialization in *A. oxylophus* and *A. aquaticus*.

KEY WORDS: lizard; habitat use; perching behavior

INTRODUCTION

Substrate type affects the locomotion of numerous animal taxa, including snakes (Kelley et al., 1997), lizards (Vanhooydonck et al., 2005; Tulli et al., 2012; Kolbe et al., 2016), terrestrial mammals (Lammers, 2007), and arboreal primates (Cartmill, 1974; Lemelin and Schmitt, 2007). Some animals demonstrate selectivity by altering their behavior to use only a narrow subset of available substrates in their habitat, whereas others have a greater breadth of substrate use that more closely reflects habitat-wide availability (Irschick and Losos, 1999). Even among close evolutionary relatives, differences in substrate availability between habitats and in animal selectivity within habitats can contribute to ecological and morphological disparity between taxa.

[1] Museum of Comparative Zoology and Department of Organismic and Evolutionary Biology, Harvard University, 26 Oxford Street, Cambridge, Massachusetts 02138, U.S.A.; e-mail: nherrmann@g.harvard.edu.

Lizards in the genus *Anolis*, or anoles, are evolutionarily closely related but can differ substantially in patterns of substrate use. In the West Indies, replicated adaptive radiations on Cuba, Hispaniola, Puerto Rico, and Jamaica have produced similar groups of habitat specialists, termed "ecomorphs" (Williams, 1972, 1983; Losos, 2009). Ecomorph species are morphologically and behaviorally adapted for locomotion in particular structural microhabitats (e.g., trunk-ground, trunk-crown, twig, grass-bush). For example, trunk-ground anoles have relatively long hindlimbs well-suited for sprinting on broad surfaces, whereas twig anoles have relatively short limbs which confer balance on narrow perches (Losos and Sinervo, 1989). About 80% of anole species in the Greater Antilles belong to an ecomorph class, with the other 20% having evolved a combination of morphology and ecology not replicated on other islands (Losos, 2009; Mahler et al., 2013).

Although West Indian ecomorphs are broadly defined by how they partition structural microhabitats, the patterns of substrate use exhibited by specialized anoles are not driven solely by selectivity. For example, a comparison of four species at one site in the Bahamas found that relative to the suite of available substrates, the trunk specialist *Anolis distichus* was selective with respect to perch diameter, length, and angle of inclination, whereas three other microhabitat specialists (trunk-ground, *Anolis sagrei*; trunk-crown, *Anolis carolinensis*; twig, *A. distichus*) were selective for only a subset of measured perch characteristics (Mattingly and Jayne, 2004). A comparison of two ecomorphs across multiple sites in Puerto Rico revealed additional complexity. The trunk-ground specialist *Anolis gundlachi* showed similar patterns of selectivity for perch diameter, angle, and visibility across sites, whereas the grass-bush specialist *Anolis*

krugi was selective about perch visibility at some sites but not at others (Johnson et al., 2006). Additionally, neither species was selective with respect to all three measured perch characteristics. Thus, the relative importance of substrate availability versus selectivity in anoles appears to depend on both species and location. However, our understanding of how substrate availability and selectivity contribute to microhabitat specialization in anoles is limited mostly to West Indian ecomorphs (e.g., Schoener, 1975; Mattingly and Jayne, 2004; Johnson et al., 2006; but see Pounds, 1988). These habitat specialists represent only one-quarter of the approximately 400 anole species that have been described (Losos, 2009).

Semiaquatic anoles, which live exclusively near streams and will sometimes enter water to feed or to escape a threat (Meyer, 1968; Campbell, 1973; Fitch, 1973; Savage, 2002), do not closely match any West Indian ecomorph in ecology and behavior. Nonetheless, they appear specialized for locomotion on particular substrates. Of the 11 semiaquatic anoles that have been described, five species are found in Central America. These Central American species are similar to each other in size, limb length, and tail morphology (Leal et al., 2002). They are also convergent in these traits with West Indian trunk-ground anoles, suggesting that they are morphologically adapted for locomotion on low, wide perches within their streamside habitats (Leal et al., 2002). Yet despite their broad morphological similarities, some Central American semiaquatic anoles differ substantially in their substrate use. In particular, *Anolis oxylophus* perches predominantly on leafy or woody vegetation and seldom on rocks (<8% of observations on rocks, Vitt et al., 1995; 0% on rocks, Muñoz et al., 2015), whereas *A. aquaticus* perches predominantly on boulders and rock walls (56.7% of observations, Muñoz et al., 2015). Furthermore, Muñoz et

Figure 1. *Anolis oxylophus* at La Selva Biological Station (left, photo by Christian Perez) and *Anolis aquaticus* at Las Cruces Biological Station (right, posed).

al. (2015) found that these species differ in two morphological traits not measured by Leal et al. (2002): claw shape, which affects clinging ability on rocks (Zani, 2000), and the ratio of fore- to hindlimb length, which is relatively large in many specialized climbers (Cartmill, 1985; Vanhooydonck and Van Damme, 2001). With its shorter claws, relatively longer forelimbs, and relatively shorter hindlimbs, *A. aquaticus* appears to be specialized for climbing on rocks, particularly when compared to *A. oxylophus*. This microhabitat specialization may be due to substrate availability, substrate selectivity by lizards, or both.

The goal of this study was to evaluate two non–mutually exclusive explanations for patterns of microhabitat specialization in *A. oxylophus* and *A. aquaticus*: First, I tested whether streams occupied by these species differ in their relative substrate availabilities. The species exhibit little to no geographic overlap (Savage, 2002). Second, I assessed whether either species selectively uses or avoids different substrates (*sensu* Johnson, 1980). Specifically, I tested whether, relative to the substrate's availability, either species shows a bias toward perching on its most commonly used substrate—wood for *A.*

oxylophus and rocks for *A. aquaticus*. I also tested whether either species selects *against* perching on the ground. Anoles are predominantly sit-and-wait foragers and spend much of their time surveying their surroundings from elevated perches (Losos, 2009). As a consequence, both ground avoidance and affinity for a non-ground substrate may contribute to patterns of substrate use in *A. oxylophus* and *A. aquaticus*.

METHODS

Study sites

I conducted this study at two forests in Costa Rica, each occupied by one species of semiaquatic anole (Fig. 1). *A. oxylophus*, the stream anole, was studied from January 9 to 11, 2016, in two shallow streams in the lowland wet forest of La Selva Biological Station (10°26′N, 83°59′W; 35 m above sea level). *Anolis aquaticus*, the water anole, was studied from January 19 to 21, 2016, in three shallow streams in the premontane wet forest of Las Cruces Biological Station (8°47′N, 82°57′W; 1,200 m above sea level). Along each stream I established a 60–140-m transect where anoles were relatively abundant.

In total, the study area in each forest consisted of 240 m of stream transect.

Lizard substrate use

I conducted visual encounter surveys along stream transects on warm, sunny days between 0700 and 1700 hours, searching for lizards perched above the stream or within 1 m of the stream's edge, where the vast majority of individuals of both species perch (Vitt et al., 1995; Eifler and Eifler, 2010). I collected data only on lizards sighted motionless from a distance of 3 m or greater to avoid including individuals responding to my presence. For each lizard, I recorded perch substrate type (woody, leafy, rocky, or ground) and, when possible, perch location along the transect, estimated relative to streamside markers that were placed every 10 m. I then attempted to catch each lizard to record its sex and measure its snout-to-vent length using digital calipers. The sex of some juvenile lizards could not be confidently identified. Across all streams I captured 75 lizards and spotted but could not capture an additional 21. Substrate use was recorded for all 96 lizards, 43 *A. oxylophus* and 53 *A. aquaticus*. All captured lizards were marked dorsally with a streak of nontoxic paint to avoid recapturing the same individual repeatedly. I noted whether uncaptured lizards were adult or juvenile based on their size, and any unmarked, similarly sized lizards subsequently observed within 10 m of that spot were excluded from the data set.

Substrate availability

To quantify substrate availability in stream transects, I divided transects into 10-m long segments, established a randomly placed plot within each segment, and measured available substrates in each plot. Each plot spanned 2 m of stream length and the entire stream width, plus 1 m of ground on

both banks. Thus, all plots were 2 m long but differed in width to standardize the area of bank at 4 m^2 per plot. Within each plot I measured the length, width, and height of every possible lizard perch up to a height of 1 m, which encompassed almost all perch heights published for individuals of both species (Vitt et al., 1995; Eifler and Eifler, 2010; Muñoz et al., 2015; this study). Substrate categories (woody, leafy, rocky, and ground) were chosen to allow comparison with the only published study to quantify substrate use in both species (Muñoz et al., 2015). I used the following criteria to define woody, leafy, and rocky perches: woody stems and logs with diameter of at least 1 cm, leaves above the ground and stream surface with dimensions of at least 5 × 12 cm, and rocks with dimensions of at least 4 × 7 × 1 cm. These criteria correspond to the smallest perches used by adult lizards I observed, suggesting that perches with these dimensions were available to lizards of all sizes. I also noted each perch's location (in the stream versus on the bank) and general orientation (e.g., for a log, whether it was lying flat or standing upright).

After data collection, I converted length, width, and height measurements to surface areas for each perch. For woody perches, I considered all exposed surface area within the plot to be available for perching, because I encountered several lizards hanging from the underside of horizontal branches and logs. For leaves, I considered only the top to be available for perching, again in accordance with my observations of lizard behavior, and treated each leaf as an ellipse. For rocks, I considered all exposed surface area available for perching and treated each rock as the upper hemisphere of an ellipsoid. To quantify the available ground area, I summed the cross-sectional area of wood and rocks laying directly on the bank and subtracted this value from 4 m^2, the total

area of bank in each plot. Having not measured leaves lying directly on the ground, this calculation of ground area does not distinguish between bare ground and ground covered by fallen leaves. In other words, "ground" in this study encompasses both exposed ground and ground covered by leaf litter.

Spatial scales for lizard substrate selectivity

Johnson (1980) distinguished between "selection," the process in which an animal chooses a resource, and "preference," the likelihood that a resource will be selected if offered on an equal basis with others. The current study was designed to measure substrate selection, not preference, because substrate availabilities were not manipulated to be equal.

Assessments of resource selection must consider spatial scales that are ecologically relevant for the organisms under study (Manly et al., 2007). In this study, one scale for substrate selection is a lizard's close vicinity (hereafter, "local scale"), represented by what a lizard perched on compared with the availability of substrates in its nearest habitat plot. However, this might not be the only scale at which substrate selection occurs. Two marked individuals in my study, one from each species, were re-sighted approximately 24 hours after initial capture at a location at least 10 m away from their original spot. A mark-recapture study on two other species of Central American semiaquatic anoles similarly found that some individuals move frequently and over distances of several meters (Campbell, 1973). Therefore, I also considered the possibility that lizards could select a particular segment along a stream transect based on its available substrates (hereafter, "stream scale"). Lizard substrate selectivity at the stream and local scales was evaluated separately.

Analyses

To test whether streams occupied by these species differ in their relative substrate availabilities, I compared streams between La Selva (*A. oxylophus*, $n = 24$ plots) and Las Cruces (*A. aquaticus*; $n = 24$ plots). I used Student's *t* tests to compare stream width and the proportional availability of each substrate (arcsine transformed). Proportional substrate availabilities in a habitat are necessarily dependent, because habitats that differ in their relative availability of one substrate inevitably differ in at least one other. Therefore, four separate *t* tests, one for each substrate, were not statistically independent, and adjusting significance values for multiple comparisons in this situation may be overly conservative. Below, I report unadjusted *P* values for these *t* tests and highlight when results become nonsignificant with sequential Bonferroni correction for multiple comparisons (Holm, 1979).

To test whether either species selectively used or avoided different substrates, I compared lizard substrate use with substrate availability at two spatial scales: the stream scale and the local scale (see the section "Spatial scales for lizard substrate selectivity"). At both scales, male, female, and unsexed juvenile lizards were pooled because they did not differ in substrate use for either species (chi-square test of independence: *A. oxylophus*: $\chi^2 = 8.35$, df = 6, $P = 0.21$; *A. aquaticus*: $\chi^2 = 6.06$, df = 6, $P = 0.42$). In two streams containing *A. aquaticus*, precise perch location along the transect was unknown because lizards were sighted before the establishment of meter markers. Therefore, substrate selection analyses were conducted only on the three streams in which each lizard could be confidently assigned to the nearest plot with known substrate availabilities—two streams for *A. oxylophus* (STR400 and STR800) and one stream for

A. aquaticus (CULV). The two streams containing *A. oxylophus* were analyzed separately because they were not identical in substrate availabilities, and a single species may exhibit different selection biases as the availability of resources changes (Johnson et al., 2006; Manly et al., 2007).

At the stream scale, I evaluated lizard substrate selectivity by testing for a relationship between the availability of particular substrates in a stream segment and the number of lizards found in that segment. In other words, were lizards more common in stream segments with certain substrates? I matched each sighted lizard to its nearest plot with known substrate availabilities, resulting in a lizard count for each plot. Separately for each stream, I used Poisson regression with a single predictor variable to test for a relationship between lizard count and the availability of a particular substrate. I fitted two models in each stream, one for lizard count against the relative availability of ground and another for lizard count against the relative availability of the most commonly used substrate for each species: wood for *A. oxylophus* and rock for *A. aquaticus*. This process of testing for correlations between lizard count and substrate availability does not quantify substrate selection in the strict sense (*sensu* Johnson, 1980) because it does not directly compare proportions of substrate use to proportions of substrate availability. Instead, it is a proxy for substrate selection predicated on the assumption, previously discussed, that lizards could select to inhabit a particular stream segment based on its available substrates.

At the local scale, I evaluated lizard substrate selectivity by comparing actual lizard substrate use to null distributions created using computer simulations. Each simulation started with lizards placed along a stream transect to match the exact locations where lizards were actually sighted. Simulated lizards were then assigned a perch, with the probability of each lizard perching on a particular substrate equal to the availability of that substrate in the lizard's closest plot. Because lizard locations along the stream were fixed to match empirical data, selection at the stream scale was effectively held constant, and simulated substrate assignments represented selection at only the local scale. I ran 20,000 simulations for each stream and stored the results to generate a distribution of lizards assigned to each substrate. These distributions represented the null hypothesis that lizards perch on a substrate in proportion to its local availability. To test whether lizards selectively chose or avoided a particular substrate, I compared the number of lizards sighted on the substrate in each stream to the simulated distribution of lizards assigned to the substrate. *P* values were calculated as the percentage of simulations that matched lizard substrate use data or exceeded it in extremity. All tests were one-tailed based on the following predictions about substrate selectivity: *A. oxylophus* is biased in favor of wood, *A. aquaticus* is biased in favor of rock, and both species are biased against ground.

When testing for resource use selectivity, it is possible to detect simultaneously multiple statistically significant selection biases, because overuse of one resource relative to its availability necessarily implies underuse of another and vice versa. To evaluate the relative strength of simultaneously detected selection biases, I conducted a second round of simulations in which I removed the statistically significant substrates one at a time and tested for the persistence of selection bias in the other substrate. For example, if *A. oxylophus* showed biases both in favor of wood and against ground, I ran two additional simulations: one with wood

TABLE 1. LIZARD SUBSTRATE USE PROPORTIONS POOLED ACROSS STREAMS FOR BOTH SPECIES AND A COMPARISON WITH OTHER STUDIES.

| Substrate | A. oxylophus | | | A. aquaticus | |
	This Study, $n = 43$	Vitt et al. 1995, $n = 87$	Muñoz et al. 2015, $n = 26$	This Study, $n = 53$	Muñoz et al. 2015, $n = 30$
Woody, %	70	59	52	32	27
Leafy, %	9	21	40	4	13
Rocky, %	5	7	0	40	57
Ground, %	16	21	8	24	3

entirely removed and another with ground entirely removed. I reduced the lizard sample size in each of these second round simulations to match the number of lizards sighted on the substrates being considered. Continuing with the *A. oxylophus* example, second round simulations addressed the following two questions:

1. Among *A. oxylophus* that did not use the ground, was there a bias in favor of wood?
2. Among *A. oxylophus* that did not use wood, was there a bias against the ground?

To estimate the probability of type II error when the null hypothesis was not rejected, I conducted a post hoc power analysis given three different effect sizes for lizard selection bias (Cohen's h: 0.2 = small, 0.5 = medium, 0.8 = large; Cohen, 1988) with sample size equal to the number of lizards sighted and alpha = 0.05. I performed all statistical analyses using R version 3.2.2 (R Development Core Team, 2015). Data and annotated R scripts are available on GitHub (Hermann, 2016).

RESULTS

The substrate use of both species in this study was broadly consistent with patterns reported in previous studies (Table 1). In particular, *A. oxylophus* was seen predominantly on woody and leafy perches and

seldom on rocky perches. In contrast, *A. aquaticus* was seen most frequently on rocks.

The streams occupied by each species differed in their substrate availabilities. Without correction for multiple comparisons, streams inhabited by *A. oxylophus* had relatively more wood ($t_{46} = 2.07$, $P = 0.043$), less rock ($t_{46} = -8.70$, $P < 0.001$), and more ground ($t_{46} = 3.78$, $P < 0.001$) than streams inhabited by *A. aquaticus* (Fig. 2). The difference in wood availability became non-significant with sequential Bonferroni correction ($P = 0.087$). Streams inhabited by each species did not differ in leaf availability ($t_{46} = 0.20$, $P = 0.84$) or in stream width ($t_{46} = 1.00$, $P = 0.32$).

Lizard substrate selectivity was analyzed at two spatial scales: the stream scale and the local scale. Analyses at both scales required matching each sighted lizard to its nearest plot with known substrate availability. The distance between a sighted lizard and its nearest plot ranged from 0 m (lizard was sighted directly in the plot) to 8 m. The mean distance within a stream ranged from 1.64 to 4.37 m (Table 2).

At the stream scale, the availability of ground in a plot did not correlate with the number of lizards sighted closest to that plot for either species (STR400: $Z = 0.14$, $P = 0.89$; STR800: $Z = -0.30$, $P = 0.76$; CULV: $Z = 1.19$, $P = 0.23$). For *A. oxylophus*, the availability of wood in a plot did not correlate with the number of lizards sighted
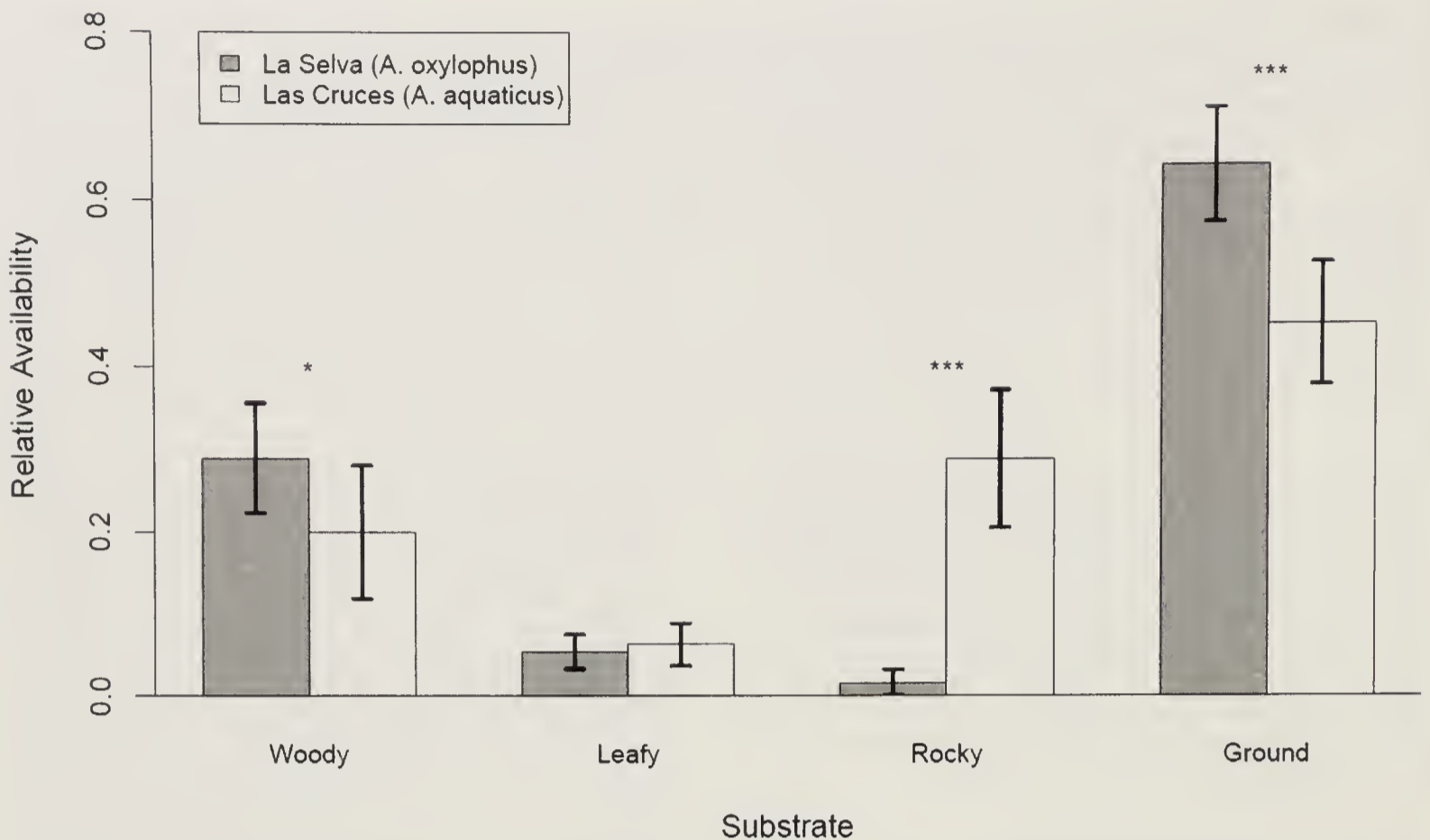
Figure 2.   Comparison of substrate availability based on surface area in streams inhabited by *Anolis oxylophus* and *Anolis aquaticus* (*n* = 24 habitat plots for each species). Error bars represent 95% confidence intervals. * *P* < 0.05 unadjusted; not significant with sequential Bonferroni correction. *** *P* < 0.001 unadjusted; still significant with sequential Bonferroni correction.

in either stream (STR400: Z = 0.53, P = 0.60; STR800: Z = 0.18, P = 0.86). For *A. aquaticus*, the availability of rocks in a plot correlated positively with the number of lizards sighted (β = 31.72, Z = 2.23, P = 0.03).

At the local scale, both species exhibited selection bias against the ground and toward either wood (*A. oxylophus*) or rocks (*A. aquaticus*; Table 3). Additional simulations were run with each of these substrates removed to test for the persistence of selection bias in the other. With the favored non-ground substrate removed from the analysis (wood for *A. oxylophus* and rocks for *A. aquaticus*), both species were still biased against perching on the ground in all streams (P < 0.01 for both species). On the other hand, with ground removed from the analysis, the biases toward wood (*A. oxylophus*) and rocks (*A. aquaticus*) disappeared (P > 0.35 for both species). Among tests that

failed to reject the null hypothesis of substrate use being equal to substrate availability, the probability of type II error varied considerably, ranging from 3% (CULV: large effect size) to 95% (STR400: small effect size; Table 3).

DISCUSSION

Two Central American semiaquatic anoles, *A. oxylophus* and *A. aquaticus*, exhibit

TABLE 2.   THE NUMBER OF LIZARDS SIGHTED AND THE MEAN DISTANCE BETWEEN LIZARDS AND THEIR CLOSEST PLOT IN EACH STREAM WHERE SUBSTRATE SELECTION WAS ANALYZED.

Species	Stream	No. of Lizards	Mean Distance to Plot (m)
A. oxylophus	STR400	22	4.37
	STR800	21	1.86
A. aquaticus	CULV	28	1.64

TABLE 3. RESULTS OF COMPUTER SIMULATIONS USED TO EVALUATE SELECTION BIAS IN LIZARD SUBSTRATE USE AT THE LOCAL SCALE. WHEN THE NULL HYPOTHESIS OF SUBSTRATE USE EQUAL TO SUBSTRATE AVAILABILITY WAS NOT REJECTED, THE PROBABILITY OF A TYPE II ERROR WAS CALCULATED ASSUMING THREE DIFFERENT EFFECT SIZES (COHEN'S $n = 0.2, 0.5, 0.8$).

Species	Stream	Substrate Excluded	No. of Lizards	H_1	P	Probability of Type II Error at Different Effect Sizes		
						Small	Medium	Large
A. oxylophus	STR400	none	22	select against ground	<0.001	—	—	—
				select for wood	<0.001	—	—	—
		wood	14	select against ground	0.003	—	—	—
		ground	16	select for wood	0.359	95%	86%	64%
	STR800	none	21	select against ground	<0.001	—	—	—
				select for wood	<0.001	—	—	—
		wood	4	select against ground	0.009	—	—	—
		ground	20	select for wood	0.462	81%	45%	30%
A. aquaticus	CULV	none	28	select against ground	<0.001	—	—	—
				select for rocks	0.047	—	—	—
		rocks	16	select against ground	0.003	—	—	—
		ground	22	select for rocks	0.420	78%	30%	3%

broadly similar morphologies adapted for locomotion on low, wide perches (Leal et al., 2002). However, they are substantially different in their use of particular substrates— *A. aquaticus* is observed most often on rocks, whereas *A. oxylophus* seldom perches on rocks (Vitt et al. 1995; Muñoz et al., 2015; this study). Slight differences in claw morphology and relative limb length between the species suggest that *A. aquaticus* is indeed a rock specialist (Muñoz et al., 2015), yet ecological and behavioral reasons for this substrate specialization had not been investigated before this study. I evaluated two non–mutually exclusive explanations for substrate specialization in *A. oxylophus* and *A. aquaticus*:

1. Stream macrohabitats occupied by these species differ in their relative substrate availabilities.
2. One or both of these species selectively use or avoid different substrates.

Results from this study provide some support for both explanations. Streams with *A. oxylophus* had almost no rocks, whereas in streams with *A. aquaticus*, rocky surfaces were the most abundant non-ground substrate. However, ground was the most commonly available substrate in all streams, a pattern that contrasts sharply with substrate use in these species, neither of which was observed on the ground often (Table 1). Consequently, differences in stream macrohabitat can only partly explain patterns of substrate use in *A. oxylophus* and *A. aquaticus*, suggesting that substrate selection is also responsible. Indeed, *A. aquaticus* was more numerous in stream segments with a higher proportion of rocky surface area. Furthermore, at the local scale, both species selected against perching on the ground and in favor of perching on their most commonly used substrate, wood for *A. oxylophus* and rocks for *A. aquaticus*. However, the results of second round simulations suggest that the bias against ground is stronger than any preference for wood or rocks. For example, whereas ground avoidance persisted in the analyses in which rocks were removed, the test for rock affinity in *A. aquaticus*, with ground removed, failed to reject the null hypothesis with only a 3% probability of type II error for a "large" effect size (Cohen's

$h = 0.8$; Cohen, 1988; Table 3). In this species, a propensity to avoid the ground, rather than to seek out rocks, probably explains its selection of substrates at the local scale. In *A. oxylophus*, the strength of ground avoidance bias relative to wood affinity is less clear, as second round tests for wood affinity had substantially less power to detect statistical significance (Table 3). In general, the post hoc power of tests for substrate selection varied because of differences in lizard sample size and stream-specific substrate availabilities. Regardless, at the local scale, both species select strongly against perching on the ground.

In both *A. oxylophus* and *A. aquaticus*, substrate selection biases at the local scale appear to contradict selection biases (or lack of biases, in the case of *A. oxylophus*) at the stream scale. In *A. aquaticus*, ground avoidance appears stronger than rock affinity at the local scale, yet at the stream scale, there is only a signal for rock affinity. *Anolis oxylophus* similarly exhibits strong ground avoidance at the local scale and no ground avoidance at the stream scale. However, a population may exhibit different habitat selection biases at different spatial scales if the factors that influence individual choice at these scales are different (Johnson, 1980; Levin, 1992; Mayor et al., 2009). Therefore, the apparent contradictions in selection biases across scales are actually not contradictory. Rather, they are suggestions that the mechanistic drivers of substrate selection at these scales are probably different and deserving of further investigation. There are several possibilities.

First, lizard distribution throughout a stream may be driven by differences in water flow. In a previous study of *A. aquaticus* living near a stream with both high-flow and low-flow segments, lizards perched near calm water more often than expected (Eifler and Eifler, 2010). Objects sitting in a stream, such as rocks and logs, slow the flow of incoming water. Thus the positive correlation between rock availability and number of *A. aquaticus* in the current study may actually reflect an affinity for low flow areas as opposed to an affinity for rocks *per se*, perhaps because lizards can escape threats more easily through less turbulent waters.

Second, some anoles actively select micro-habitats to stay within a certain range of body temperatures (Hertz, 1992), either with (Hertz et al., 1994) or without (Vitt et al. 2001) using light intensity as a cue. Anecdotal evidence indicates that *A. oxylophus* actively seeks out shade (Savage, 2002). Shaded areas are likely to have a high availability of upright woody perches that support canopy cover, but sunny areas may also have high wood availability in the form of fallen logs and branches, potentially explaining the lack of correlation between wood availability and stream-wide spatial distribution in this species.

Third, patterns of habitat use in *A. oxylophus* and *A. aquaticus* may be partly driven by other characteristics of structural micro-habitat besides substrate type, such as perch height and perch width, which together describe the primary niche axis for diversification in West Indian ecomorphs (Williams, 1972, 1983). The clearest substrate selection bias detected in this study is ground avoidance at the local scale for both species. However, because ground is by definition at a perch height of zero, *A. oxylophus* and *A. aquaticus* may avoid the ground to circumvent a particular substrate (e.g. sand or mud) or elevate their perch height irrespective of substrate. The latter is more likely for two reasons. First, almost all anoles survey their surroundings from elevated perches, even though the ground is a widely available substrate (reviewed in Losos, 2009). Second, in this study, leaves were available for perching both on the ground and on elevated

branches. The former were classified as part of "ground," whereas the latter were classified as "leaves." Additional analyses revealed that neither *A. oxylophus* nor *A. aquaticus* exhibited a selection bias for elevated leaves relative to their local availability in this study (two-tailed test, $P > 0.05$ for all three streams). Thus these lizards do not appear to avoid leafy substrates *per se*, but they still avoid ground which is sometimes covered in leaves, further suggesting that ground avoidance is more likely due to perch height elevation than to circumvention of a particular substrate.

Regardless of what drives substrate selection in these species, selection biases at both the stream scale and local scale, along with substrate availability, all contribute to microhabitat specialization in *A. oxylophus* and *A. aquaticus*. Surface area for perching in all streams was dominated by the ground, but both species selectively avoided the ground relative to its local availability and perched instead on elevated substrates. These elevated substrates were different depending on the stream—predominantly wood for *A. oxylophus* and rocks for *A. aquaticus*. In *A. aquaticus*, the tendency for more individuals to inhabit stream segments with the highest availability of boulders and rock walls further increases their exposure to rocky surfaces. This may partly explain why *A. aquaticus* has claw and limb morphology specialized for locomotion on rocks, whereas *A. oxylophus* is more similar to other Central American semiaquatic anoles *A. lionotus* and *A. poecilopus*, which appear to be stream side substrate generalists (Muñoz et al., 2015).

As is true with several West Indian species (Mattingly and Jayne, 2004; Johnson et al., 2006), both substrate availability and lizard substrate selectivity appear to contribute to microhabitat specialization in *A. oxylophus* and *A. aquaticus*. Future work should evaluate these forces in other mainland anoles, particularly the other Central American semiaquatic anoles *Anolis poecilopus* and *Anolis lionotus*. These species are closely related to *A. oxylophus* and thus together may represent a single evolutionary transition to a semiaquatic lifestyle (Nicholson et al., 2005). Additionally, why semiaquatic anoles are abundant in some streams and not others has yet to be investigated, although differences in predation pressure likely play a role (Vitt et al., 1995). As population density changes, patterns of habitat selection are likely to change as well (Greene and Stamps, 2001). Lastly, anoles are an excellent system for studying if and how substrate selectivity changes during particular activities or behaviors. Several species are known to engage in different behaviors at different perch heights (Andrews, 1971; Paterson, 1999; Kamath et al. 2013), suggesting that patterns of substrate selectivity may change depending on whether an animal is foraging, displaying, or merely scanning its surroundings. Longer and repeated observations of individuals, combined with measures of substrate availability, may reveal complex interactions between habitat structure and adaptive behaviors.

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